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(54) Title: A MOLECULAR MARKER FOR THE GENE DETERMINING THE FRUCTOSE TO GLUCOSE RATIO IN MATURE TOMATO FRUIT

(57) Abstract

A molecular marker for a gene determining fructose to glucose ratio in mature tomato fruit. In accordance with a preferred embodiment of the present invention, the marker includes a first amplification product generated by a primer called an MS6 primer, the MS6 primer including a nucleotide sequence TCTCTCTCTCTCTCCC.

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A MOLECULAR MARKER FOR THE GENE DETERMINING THE FRUCTOSE TO GLUCOSE RATIO IN MATURE TOMATO FRUIT

FIELD OF THE INVENTION

The present invention relates generally to a method of breeding tomatoes having superior taste characteristics and to tomatoes having superior taste characteristics, and particularly to a molecular marker for the gene determining the fructose to glucose ratio in mature tomato fruit.

BACKGROUND OF THE INVENTION

Taste characteristics are a major determinant of fruit quality for both processing and fresh market tomatoes (see Stevens, M.A. 1986. Inheritance of tomato fruit quality components. Plant Breeding Reviews 4: 274-310). One of the major components of taste in tomatoes is soluble sugar content. The soluble sugar content of all known commercial cultivars of tomatoes (*Lycopersicon esculentum* Mill.) primarily includes the hexose sugars glucose and fructose in near-equimolar ratios (1:1 to 1:1.3) (see Davies J.N. and Hobson G.E. 1981. The constituents of tomato fruit- the influence of environment, nutrition and genotype, CRC Critical Review Food Science and Nutrition, 15:205-280; Davies J.N. and Kempton, R.J. 1975. Changes in the individual sugars of tomato fruit during ripening. J. Sci. Fd. Agric. 26: 1103-1110). In commercial *Lycopersicon esculentum* cultivars the disaccharide sucrose is also present but at concentrations rarely exceeding 0.5% on a fresh weight basis. Certain wild species of *Lycopersicon*, such as *L. hirsutum*, accumulate high concentrations of sucrose, which may reach 4% on a fresh weight basis (see Miron, D. and Schaffer, A.A. 1991. SPS, SS and invertase activities in developing fruit of *Lycopersicon esculentum* and the sucrose accumulating *L. hirsutum*. Plant Physiol. 95: 623-627). In the presence of high sucrose, these fruit accumulate low levels of the hexoses fructose and glucose, typically less than 1% each on a fresh weight basis (Davies J. N. On the Occurrence of Sucrose in *Lycopersicon* Fruit and its Nature, Nature, Vol. 266, 586-587, 1966). However, in these fruit the ratio of fructose to glucose is unusually high, more than 1.5:1.

Typically, plant breeders seek to improve the sweetness component of tomato flavor by increasing total soluble solids (TSS), measured by refractometric determination of a sample of juice and expressed as Brix. This measurement however does not differentiate between the component sugars. Fructose is significantly sweeter than both glucose and sucrose (see Biester, A.M., 1925. Carbohydrate studies: I. Relative sweetness of pure sugars. Amer. J. Physiology

73: 387-400). giving a tomato with a relatively high fructose content distinct advantages in terms of superior taste characteristics.

Tomatoes with high fructose to glucose ratios have been developed, using a method of selection described in applicant/assignee's Israel patent application 105243, PCT patent application PCT/US94/03522 and US patent application 08/530,216, the disclosures of which are incorporated herein by reference. In summary, this method consists of hybridizing a tomato plant of the *L. esculentum* species with a plant of the *L. hirsutum* species and in the subsequent progenies selection of mature fruit with fructose/glucose ratios of more than 1.8, together with fructose levels more than 1.3% on a fresh weight basis. The analysis of mature fruit sugars in the described method is via direct chemical analysis of the fruit sugars, for example by chromatographic separation of individual sugars.

Molecular markers have been used as a method of selection in plant breeding, with obvious advantages (see Tanksley, S.D., Ganal, M.W., Prince, J.P. et al. 1992. High density molecular linkage maps of the tomato and potato genomes. Genetics, 132: 1141-1160; Williamson V.M., Ho J.-Y., Wu F.F., Miller N. and Kaloshian I.. 1994. A PCR-based marker tightly linked to the nematode resistance gene, Mi, in tomato. Theor. Appl. Genet., 87:757-763; Chagu'e V., Mercier J.C., Gu'enard M., de Courcel A., and Vedel F. 1996. Identification and mapping on chromosome 9 of RAPD markers linked to Sw-5 in tomato by bulked segregant analysis. Theor. Appl. Genet., 92:1045-1051). Several strategies to modulate sugar concentration and profile in ripe tomato fruit have been explored, including genetic approaches. However, precision breeding towards such directions involves assessment of reducing sugars carried out by HPLC (high pressure liquid chromatography) that is expensive and time consuming. DNA markers could potentially alleviate this problem, enabling the identification and selection of genetic material at the seedling stage, thus reducing significantly effort and time. During recent years, international efforts were invested aiming at the genome mapping of several plant species such as the tomato, potato and maize, using DNA markers (see Helentjaris T., Slocum M., Wright S., Schaefer A. and Neinhuis J. 1986. Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor. Appl. Genet., 72: 761-769; Tanksley et al., 1992). Apart from being an efficient tool for many breeding and genetic analyses (reviewed by Hillel J., Dunnington , E.A, and Siegel P.B. 1992. DNA markers in poultry breeding and genetic analysis. Poult. Sci. Rev., 4:169-186), DNA markers also provide starting points for cloning genes of interest. Recently, there were several successful reports of gene isolation in higher plants by positional cloning (reviewed by

Tanksley, S.D., Ganal, M.W. and Martin, G.B. 1995. Chromosome landing: a paradigm for map-based cloning in plants with large genomes. Trends Genet., 11: 63-68).

Molecular linkage maps are largely composed of restriction fragment length polymorphism (RFLP) markers. RFLP markers require a cloned probe, endonuclease digestion of genomic DNA and time consuming DNA transfer, labeling and hybridization steps. At the end of this laborious process only a single locus of a very limited polymorphic content is usually revealed. More efficient polymorphism assays can be obtained from multilocus DNA probes yielding DNA fingerprints (DFP, see Jeffreys A.J., Wilson V. and Thein S.L. 1985 a. Hypervariable minisatellite regions in human DNA. Nature, 314: 67-73; Jeffreys A.J., Wilson V. and Thein S.L. 1985 b. Individual-specific fingerprint of human DNA. Nature, 316: 76-79) and the polymerase chain reaction (PCR, see Saiki R.K., Scharf S., Faloona F.A., Mullis K.B., Horn G.T., Erlich H.A. and Arnheim N. 1985. Enzymatic amplification of b-globin sequences and restriction site analysis for the diagnosis of sickle cell anemia. Science, 230:1350-1354). Polymorphism obtained by DFP is very high and therefore considered very useful for various breeding and genetic applications (see Hillel et al., 1992). DNA fingerprints were shown to be applicable for the detection of genetic association with agriculturally important traits (see Plotsky Y., Cahaner A., Haberfeld A., Lavi U. and Hillel J. 1990. Analysis of Genetic Association between DNA fingerprint bands and quantitative traits by DNA mixes. Proceedings of the 4th World Congress on Genetics Applied to Livestock Production 13: 133-136. Edinburgh), for efficient gene introgression programs (see Hillel J., Schaap T., Haberfeld A., Jeffreys A.J., Plotzky Y., Cahaner A. and Lavi U. 1990. Genomic selection: application of DNA fingerprints for efficient gene introgression. Genetics, 124:783-789) and for genetic and evolutional analyses (see Lavi U., Hillel J., Vainstein A., Lahav E., Sharon D. 1991. Application of DNA fingerprints for identification and genetic analysis of avocado (*Persea americana*). J. Amer. Soc. Hort. Sci., 116:1078-1081). Several PCR-based marker identification techniques were also developed and found useful in the detection of DNA sequences linked to genes of interest. These techniques include the random amplified polymorphic DNA (RAPD, see Williams J.G.K., Kublik A.R., Livak K.J., Rafalski J.A. and Tingey S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res., 18: 6531-6535), microsatellite or simple sequence repeat analysis (SSR, see Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucl. Acids Res., 17: 6463-6471), inter SSR polymorphism using single primers of simple sequence repeats (see Gupata M., Chyi Y.-S., Romero-Severson J.

and Owen J.L. 1994. Amplification of DNA markers from evolutionarildiverse genomes using single primers of simple-sequence repeats. Theor. Appl. Genet., 89:998-1006) and the recently developed technique of amplified restriction fragment polymorphism analysis (AFLP, see Zabeau M. and Vos P. 1993. Selective restriction fragment amplification: a general method for DNA fingerprinting. European Patent Application 92402629.7 (Publication number: 0 534 858 A1)). The PCR techniques, mentioned above, can detect more subtle sequence polymorphisms than RFLP analysis and require only a small amount of DNA. RAPD and inter SSR analysis is low cost and easy to perform because no prior target DNA sequence information in polymorphic DNA regions is required for its implementation. AFLP is more expensive to produce but has the capacity to detect a much greater number of polymorphic loci in a single assay than other currently available PCR-based techniques. Microsatellites, on the other hand, are expensive to produce since they require allele specific primers and detect only a single polymorphic locus in a single assay.

In the case of selection for sugar content of mature fruit, a molecular marker has the advantage of allowing for selection at the young seedling stage , in contrast to selection only at the mature fruit stage. Furthermore, selection using a molecular marker eliminates the confounding effects of environmental influences on the plant phenotype which can limit the effectiveness of selection for a phenotypic trait such as mature fruit sugar content.

SUMMARY OF THE INVENTION

The present invention seeks to provide a molecular marker for a gene determining fructose to glucose ratio in mature tomato fruit. The marker can be used to find this gene and produce tomato seeds, plants and/or fruit with the desirable characteristic of increased fructose to glucose ratio.

There is thus provided in accordance with a preferred embodiment of the present invention a molecular marker for a gene determining fructose to glucose ratio in mature tomato fruit.

In accordance with a preferred embodiment of the present invention the marker includes a first amplification product generated by a primer called an MS6 primer, the MS6 primer including a nucleotide sequence TCTCTCTCTCTCTCCC.

Further in accordance with a preferred embodiment of the present invention the marker includes a fragment having a nucleotide sequence as follows:

1 TCTCTCTCTC TCTCTCCCTA AATATCTTAT CAATTGCTGA AGAACTCTAT

51 ATATGGCCGA TCCACCACCG GCGGAAAGAT GTGAACTTCA TCACGTAATC

101 GATGGTCTTG CAGCTATACC TCTTGACCGT ACTTTTTACG CCCTACACAT

151 CATCACAACA TACCAAGAAA ATTTTGAAGA TTTCCTATGG AATTTCAAAA

201 TGTTAATTTG ACAACCGATG CCATCATTCT TGATAAATCA TTTAATGATA

251 ATTTTTCAAA CCCTATCAAT GATGATGCAT TTCAAAACCC ATAATCTACC

301 AGACTGTCGC GGGTCGGAGT AAGGTTTTGA CTCAAATCAA ATGAACCCAA

351 CTAGCTTTTT TTTATTGAAT TTTATGAAAA ACATCTTGAA AGAGTTTTGA

401 CTATTGACGG TCATGTGAAT GGGACTTATT GTTCTGGGAA ATACGGTGGT

451 AAAGATCATA GCTCCCATTG TTGGTGGGGA GATCAGGGTG AAAGCAATAT

501 GAAAAATACC AATTAGTTTG CAGATGAGTT TACTCATATG GGCAATGGCA

551 CTGATCCTCT GGAAGCCTCT CTGTATGTCC CAGGGAATGA TAAACTTGTT

601 CAGATTGATG GGAAGTTGAT AATTCAATCC GTATTGGCAA GTGAGAAAGC

651 CATGGTATTT CATGGAAGTG CTCATAAGAA AAATAGAGAG TTAGGCCTCA

701 CAGGTGATTT AGCCCCTACT ATCCCAGGAA TCCATCCTCA CCTTTATCAA

751 AGTCCTGCAA TGAGACAAAG CATTTCTTGC ATATGAGGGG AGAGAGAGAG

801 AGAGA .

Still further in accordance with a preferred embodiment of the present invention the first amplification product is allelic to a second amplification product obtained by a primer called an MS8 primer, the MS8 primer including a nucleotide sequence TCTCTCTCTCTCTCCG.

There is also provided in accordance with a preferred embodiment of the present invention a method for breeding tomato plants that produce tomatoes having superior taste characteristics, including the steps of crossing at least one *Lycopersicon esculentum* plant with a *Lycopersicon* spp. to produce hybrid seeds, collecting the hybrid (F_1) seeds, growing plants from the F_1 seeds, pollinating the F_1 plants, collecting the hybrid seeds produced by the F_1 plants, growing plants from the seeds produced by the F_1 plants, measuring glucose and fructose content of ripe fruit produced from the plants grown from the seeds of the F_1 plants, providing at least one marker for a gene which provides increased fructose to glucose ratio in a tomato plant, and using the at least one marker to select a tomato plant with tomato fruit having desired characteristics including a fructose to glucose ratio greater than a ratio of standard *Lycopersicon esculentum*.

There is also provided in accordance with a preferred embodiment of the present invention a method for finding a gene that produce tomatoes having superior taste

characteristics, including the steps of providing at least one marker for a gene which provides increased fructose to glucose ratio in tomato plants, and using the at least one marker to find the gene.

In accordance with a preferred embodiment of the present invention the method further includes cloning the gene.

Additionally in accordance with a preferred embodiment of the present invention the method includes the step of propagating the plants with tomato fruits having the desired characteristics. Alternatively the plants may be propagated by vegetative propagation or by seed.

A tomato plant, tomato fruit and/or tomato seed may be produced in accordance with any of the methods of the present invention.

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

Reference is now made to a method for selecting, in a breeding program , tomato plants with the genetic composition that determines that the mature fruit will have a fructose to glucose ratio of over 1.5:1. The method of developing the plant material is as described in applicant/assignee's PCT patent application PCT/US94/03522. Reference is now made to the following example which illustrates the invention.

Plant material description and analysis of sugar content in mature fruit

Two parental lines of *Lycopersicon esculentum* differing significantly in their fructose to glucose ratio in the mature fruit were selected for this study, together with F_1 and F_2 populations generated by crossing the two parental lines. The high fructose to glucose ratio breeding line was derived from the introgression of the trait of high fructose to glucose ratio from the wild species *Lycopersicon hirsutum* (LA1777), as described in PCT patent application PCT/US94/03522.

The following procedure was carried out for soluble sugar determination. Fruit portions of about 500 mg fresh weight were placed in 80% ethanol and soluble sugars were extracted from the tissue by heating to 70°C, as described in Miron and Schaffer (1991). Sugars were chromatographically separated by HPLC using a Bio-Rad Fast Carbohydrate column according to manufacturer's directions, as in Miron and Schaffer (1991). Sucrose glucose and fructose were identified by their retention times, refractometrically, and quantified in comparison to sugar standards.

Description of the PCR method, the MS6 and MS8 marker and the tail-end analysis

Genomic DNA was extracted from the 2 parental lines with divergent fructose to glucose ratio in the mature fruit and from individual plants of the F_1 and F_2 populations generated by crossing the two parental lines. The individual plants from the F_2 population segregated for the trait of fructose to glucose ratio, the range being 1-3.75. Individual plants from the F_2 population could therefore be easily ranked for the trait of fructose to glucose ratio. The genomic DNA was extracted as in Fulton, T.M., Chunwongse, J. and Tanksley, S.D. 1995. Microprep protocol for extraction of DNA from tomato and other herbaceous plants. Plant Molecular Biology Reporter 13: 207-209. In short, 50-100 mg of leaf tissue was ground in the presence of 2.5 parts DNA extraction buffer (0.35 M sorbitol, 0.1 M Tris-base, 5 mM EDTA, pH, 7.5); 2.5 parts nuclei lysis buffer (0.2 M Tris, 0.05 M EDTA, 2 M NaCl, 5% CTAB); 1 part 5% sarkosyl and 0.3 gm sodium bisulfite/100 ml. After incubation at 65 C for 120 min DNA was extracted with chloroform:isoamyl (24:1), precipitated with isopropanol, washed with 70% ethanol, dried and resuspended in ddH_2O.

A collection of 18 base pairs single strand DNA primers containing di- and tri - nucleotide repeats were synthesized (Pharmacia Biotech, Inc., Austria) and used in the presence of template DNA to screen by a polymerase amplification reaction DNA sequences linked to the gene encoding high fructose to glucose ratio. Initially, DNA samples extracted from individual plants from the two parental lines were used to identify, by an amplification reaction, polymorphic DNA patterns.

Amplification reactions (25 ml final volume) contained 10 ng template DNA, 25 mM TAPS (pH 9.3 at 25°C), 50 mM KCl, 2mM MgCl2, 1 mM β-mercaptoethanol, 0.2 mM of each of the four deoxyribonucleotide triphosphates (dATP, dCTP, dGTP and dTTP), 20 ng of a single primer (or 10 ng of each of two primers when using a combination of two primers), and 1 unit of thermostable Taq DNA polymerase (SuperNova Taq Polymerase, MADI LTD., Israel). Rmixtures were overlaid by 15 ml of light mineral oil, and reactions were carried out in an automated thermocycler (MJ Research Inc., Watertown, Massachusetts, USA). Initial incubation was at 94°C for 1.5 min, followed by 34 cycles of denaturation at 94°C for 1 min, annealing at 45°C for 1 min, and polymerization at 72°C for 1 min. Final polymerization at 72°C was carried out for 7 min after cycles were completed. The amplification products were visualized by electrophoresis in 1.5% agarose gels and detected by staining with ethidium bromide.

Five polymorphic DNA bands were identified in the initial selection using the DNA extracted from the parental lines in the amplification reactions. Three of the bands appeared

solely in the amplification pattern of DNA samples extracted from individual plants of the parental lines characterized by high fructose to glucose ratio and two of the bands appeared solely in the amplification pattern of DNA samples extracted from individual plants of the parental lines characterized by low fructose to glucose ratio. Theses five bands were further characterized in amplification products obtained using DNA samples extracted from the F_2 populations. Initially, tail analysis of DNA mixes extracted from 10 plants characterized by the highest fructose to glucose ratios and 10 plants characterized by the lowest fructose to glucose ratios was carried out. Based on the tail analysis, two amplification products were selected for further study. One amplification product, 805 bp in size, that was generated by a primer termed MS6, appeared to be associated in coupling to the trait of high fructose to glucose ratio. The other amplification product, 1000 bp in size, was generated by a primer termed MS8 appeared to be associated in repulsion to the trait of high fructose to glucose ratio. The nucleotide sequences of these primers were: MS6 = TCTCTCTCTCTCTCTCCC, MS8 = TCTCTCTCTCTCTCTCCG.

Inheritance of the markers in segregating population; inheritance of the trait

These MS6 and MS8 primers were further used to analyze individual DNA samples extracted from the entire F_2 population. The analysis of the amplification products obtained from DNA samples extracted from individual F_2 plants revealed that the product obtained by the MS6 primer was allelic to the amplification product obtained by the MS8 primer. Therefore, individual plants from the entire F_2 population could be characterized as homozygous to the marker allele generated by MS6 that is associated with the trait of high fructose to glucose ratio, homozygous to the marker allele generated by MS8 that is associated with low fructose to glucose ratio and heterozygous.

Genotype-phenotype relation

Analyses of variance were carried out using results obtained from the F_2 population to determine the effect of association between each of the marker bands or their combination (zygosity) and the trait of fructose to glucose ratio and the percentage of fructose to glucose variation explained by these variation components. The DNA markers obtained were found highly and significantly associated with the trait of fructose to glucose ratio (Table 1). The association between the zygosity status and the trait of fructose to glucose ratio was highly significant at a high log-of-differences (LOD) score explaining 40.5% of the total variation in fructose to glucose ratios observed in the F_2 population (Table 1).

A nested analysis of variance was also carried out to determine the total variation explained by the zygosity status of F_2 plants, plants within zygosity status and fruits within plants (Table 2). The zygosity status appeared to be highly significant in this analysis as well (10 < LOD score < 11) and the total variation explained by the 3 variation components reached a very high and statistically significant value of 75.4%.

Similar analyses carried out on the components of fructose to glucose ratio (i.e. fructose and glucose levels) were found statistically insignificant. In conclusion, the results presented suggest that: 1. The DNA markers obtained by the amplification reactions using MS6 and MS8 primers are highly associated with a major gene encoding fructose to glucose ratios in the mature tomato fruits; and

2. The gene identified can directly modulate fructose to glucose ratios without an apparent effect on the components of this ratio i.e., fructose and glucose levels.

Table 1. Association between the DNA markers generated by MS6 and MS8 primers and the trait of fructose to glucose ratio obtained in the F_2 population.

Source of variation	Prob>F	LOD Score	Variation explained (%)
MS6 allele	5.86×10^{-7}	5<LOD<6	24.6
MS8 allele	1.94×10^{-8}	6<LOD<7	30.0
Zygosity	1.21×10^{-10}	8<LOD<9	40.5

Table 2. Nested analysis of variance to determine the effect of principle variance components in the F_2 population.

Source of variation	Mean square	F ratio	Prob>F
Zygosity	9.9598	33.52	1.48×10^{-11}
Plants (zygosity)	0.2984	3.68	7.4×10^{-14}
Fruits (plants)	0.0811	-	-

Genetic effect

The genetic effect of the gene encoding fructose to glucose ratio could be estimated based on the DNA markers amplified by the MS6 and MS8 primers. The allele encoding high fructose to glucose ratio appeared to be partially dominant.

Table 3. Effect of the gene encoding fructose to glucose (f/g) ratio in the mature tomato fruit of segregating F_2 population.

Genotype	F/G Ratio

MS6 MS6	1.92 ±0.12
MS6 MS8	1.47 ±0.05
MS8 MS8	1.15 ±0.02

Sequence of 805 bp fragment generated by the inter SSR primer MS6

The DNA fragment amplified by the MS6 primer was extracted from the agarose gel using the Geneclean II kit (BIO 101, Vista CA, USA) and cloned using the p-GEM easy vector cloning system (Promega corporation, Madison WI, USA). The nucleotide sequence of the cloned fragment was determined using an ABI PRISM 377 DNA sequencer and is as follows:

1 TCTCTCTCTC TCTCTCCCTA AATATCTTAT CAATTGCTGA AGAACTCTAT
51 ATATGGCCGA TCCACCACCG GCGGAAAGAT GTGAACTTCA TCACGTAATC
101 GATGGTCTTG CAGCTATACC TCTTGACCGT ACTTTTTACG CCCTACACAT
151 CATCACAACA TACCAAGAAA ATTTTGAAGA TTTCCTATGG AATTTCAAAA
201 TGTTAATTTG ACAACCGATG CCATCATTCT TGATAAATCA TTTAATGATA
251 ATTTTTCAAA CCCTATCAAT GATGATGCAT TTCAAAACCC ATAATCTACC
301 AGACTGTCGC GGGTCGGAGT AAGGTTTTGA CTCAAATCAA ATGAACCCAA
351 CTAGCTTTTT TTTATTGAAT TTTATGAAAA ACATCTTGAA AGAGTTTTGA
401 CTATTGACGG TCATGTGAAT GGGACTTATT GTTCTGGGAA ATACGGTGGT
451 AAAGATCATA GCTCCCATTG TTGGTGGGGA GATCAGGGTG AAAGCAATAT
501 GAAAAATACC AATTAGTTTG CAGATGAGTT TACTCATATG GGCAATGGCA
551 CTGATCCTCT GGAAGCCTCT CTGTATGTCC CAGGGAATGA TAAACTTGTT
601 CAGATTGATG GGAAGTTGAT AATTCAATCC GTATTGGCAA GTGAGAAAGC
651 CATGGTATTT CATGGAAGTG CTCATAAGAA AAATAGAGAG TTAGGCCTCA
701 CAGGTGATTT AGCCCCTACT ATCCCAGGAA TCCATCCTCA CCTTTATCAA
751 AGTCCTGCAA TGAGACAAAG CATTTCTTGC ATATGAGGGG AGAGAGAGAG
801 AGAGA

The underlined are annealing sites of the MS6 primer used to identify the marker linked to fructose to glucose ratio. It is possible that the amplification product generated by the MS6 primer may be part of, in the area of, or lead to finding the gene that determines the increased fructose to glucose ratio.

CLAIMS

What is claimed is:

1. A molecular marker for a gene determining fructose to glucose ratio in mature tomato fruit.

2. A marker according to claim 1 and comprising a first amplification product generated by a primer called an MS6 primer, said MS6 primer comprising a nucleotide sequence TCTCTCTCTCTCTCTCCC.

3. A marker according to claim 2 and comprising a fragment having a nucleotide sequence as follows:

1 TCTCTCTCTC TCTCTCCCTA AATATCTTAT CAATTGCTGA AGAACTCTAT
51 ATATGGCCGA TCCACCACCG GCGGAAAGAT GTGAACTTCA TCACGTAATC
101 GATGGTCTTG CAGCTATACC TCTTGACCGT ACTTTTTACG CCCTACACAT
151 CATCACAACA TACCAAGAAA ATTTTGAAGA TTTCCTATGG AATTTCAAAA
201 TGTTAATTTG ACAACCGATG CCATCATTCT TGATAAATCA TTTAATGATA
251 ATTTTTCAAA CCCTATCAAT GATGATGCAT TTCAAAACCC ATAATCTACC
301 AGACTGTCGC GGGTCGGAGT AAGGTTTTGA CTCAAATCAA ATGAACCCAA
351 CTAGCTTTTT TTTATTGAAT TTTATGAAAA ACATCTTGAA AGAGTTTTGA
401 CTATTGACGG TCATGTGAAT GGGACTTATT GTTCTGGGAA ATACGGTGGT
451 AAAGATCATA GCTCCCATTG TTGGTGGGGA GATCAGGGTG AAAGCAATAT
501 GAAAAATACC AATTAGTTTG CAGATGAGTT TACTCATATG GGCAATGGCA
551 CTGATCCTCT GGAAGCCTCT CTGTATGTCC CAGGGAATGA TAAACTTGTT
601 CAGATTGATG GGAAGTTGAT AATTCAATCC GTATTGGCAA GTGAGAAAGC
651 CATGGTATTT CATGGAAGTG CTCATAAGAA AAATAGAGAG TTAGGCCTCA
701 CAGGTGATTT AGCCCCTACT ATCCCAGGAA TCCATCCTCA CCTTTATCAA
751 AGTCCTGCAA TGAGACAAAG CATTTCTTGC ATATGAGGGG AGAGAGAGAG
801 AGAGA .

4. A marker according to claim 2 wherein said first amplification product is allelic to a second amplification product obtained by a primer called an MS8 primer, said MS8 primer comprising a nucleotide sequence TCTCTCTCTCTCTCTCCG.

5. A marker according to claim 3 wherein said first amplification product is allelic to a second amplification product obtained by a primer called an MS8 primer, said MS8 primer comprising a nucleotide sequence TCTCTCTCTCTCTCTCCG.

6. A method for breeding tomato plants that produce tomatoes having superior taste characteristics, comprising the steps of:

crossing at least one *Lycopersicon esculentum* plant with a *Lycopersicon* spp. to produce hybrid seeds;

collecting the hybrid (F_1) seeds;

growing plants from the F_1 seeds;

pollinating the F_1 plants;

collecting the hybrid seeds produced by the F_1 plants;

growing plants from the seeds produced by the F_1 plants;

measuring glucose and fructose content of ripe fruit produced from the plants grown from the seeds of the F_1 plants;

providing at least one marker for a gene which provides increased fructose to glucose ratio in a tomato plant; and

using said at least one marker to select a tomato plant with tomato fruit having desired characteristics including a fructose to glucose ratio greater than a ratio of standard *Lycopersicon esculentum*.

7. A method for finding a gene that produce tomatoes having superior taste characteristics, comprising the steps of:

providing at least one marker for a gene which provides increased fructose to glucose ratio in tomato plants; and

using said at least one marker to find said gene.

8. A method according to claim 7 and further comprising cloning said gene.

9. A method according to claim 6 and additionally comprising the step of propagating said plants with tomato fruits having the desired characteristics.

10. A method according to claim 9 wherein the step of propagating includes the step of vegetative propagation.

11. A method according to claim 9 wherein the step of propagating includes the step of propagation by seed.

12. A tomato plant produced according to the method of claim 6.

13. A tomato fruit produced by a tomato plant in accordance with claim 12.

14. A tomato seed which when grown yield a tomato plant in accordance with claim 12.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL98/00336

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01H 5/00, 1/04, 5/08; C12N 5/14; C12P 19/12
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 800/200, 220, 255, DIG. 44; 435/100, 172.2, 421, 423; 47/58, DIG. 1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAB, AGRICOLA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,E	US 5,817,913 A (SCHAFFER) 06 October 1998, see entire document.	1-14
Y	US 5,434,344 A (BENNETT et al.) 18 July 1995, col. 3, lines 16-25 and col. 4, lines 40-68.	1-14
Y,P	US 5,750,869 A (SHEWMAKER) 12 May 1998, col. 2, lines 25-33 and 50-55, col. 3, lines 16-35.	1-14
Y	TANKSLEY et al. Use of molecular markers in breeding for soluble solids content in tomato - a re-examination. Theoretical and Applied Genetics. 1988, Vol. 75, pages 811-823, especially page 822.	1-14

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
07 OCTOBER 1998	28 OCT 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MELISSA KIMBALL Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL98/00336

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	STOMMEL et al. Genetic control of fruit sugar accumulation in a Lycopersicon esculentum x L. hirsutum cross. Journal of the American Society of Horticultural Sciences. 1993, Vol. 118, No. 6, pages 859-863, see entire article.	1-14
Y	CHETELAT et al. Inheritance and genetic mapping of fruit sucrose accumulation in Lycopersicon chmielewskii. The Plant Journal. 1993, Vol. 4, No. 4, pages 643-650, see entire article.	1-14

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER:
US CL : 800/200, 220, 255, DIG. 44; 435/100, 172.2, 421, 423; 47/58, DIG. 1